Eur J Vasc Endovasc Surg **35**, 644–651 (2008)
doi:10.1016/j.ejvs.2008.02.003, available online at http://www.sciencedirect.com on ScienceDirect

Deferred Urgency Carotid Artery Stenting in Symptomatic Patients: Clinical Lessons and Biomarker Patterns from a Prospective Registry

C. Setacci,[1*] G. de Donato,[1] E. Chisci,[1] F. Setacci,[1] A. Stella,[2] G. Faggioli,[2] B. Reimers,[3] C. Cernetti,[3] M.J. Lopera Quijada,[4] B. Cappi[4] and G. Sangiorgi[5] on behalf of the Submarine Registry Group

[1]*Vascular and Endovascular Surgery Unit – University of Siena, Siena, Italy,* [2]*Vascular Surgery Unit – University of Bologna, Bologna, Italy,* [3]*Cardiology Department, Mirano Hospital, Mirano (Venice), Italy,* [4]*Abbott Vascular Knoll-Ravizza SpA, Milan, Italy, and* [5]*Invasive Cardiology Unit, San Raffaele Scientific Institute, Milan, Italy*

Introduction. *The aim of this prospective observational registry was to study the outcome of symptomatic patients presenting with recent TIA or minor stroke and severe carotid stenosis, submitted to early percutaneous treatment by stenting. A secondary aim was to evaluate the biological activity of the symptomatic carotid plaques by serial serum and urinary markers (PAPP-A, hs-CRP, MMP-2/MMP-9, IL-6/IL-8, TNF alpha, CD40L) measured by enzyme-linked immunosorbent assay before and after treatment.*
Methods. *From May 2005 to June 2006, 57 patients were enrolled in this prospective registry. All patients underwent carotid stenting using a concentric filter for cerebral protection. The procedure was performed within 24–48 hrs of the last attack in patients with TIA (*n = *24, 42%) and between 14 and 30 days in patients with stroke (*n = *33, 58%).*
Results. *Successful stent implantation was achieved in all cases (100%). Adverse events at 1 month were 1 death (1.7%) and 2 TIAs (3.5%). Some of the vulnerability markers, in particular those reflecting an active systemic inflammatory process of the plaque (PAPP-A, hs-CR, and IL-6), were significantly elevated at the time of enrolment, increased after stenting and decreased after 30 days.*
Conclusion. *Deferred CAS is feasible and safe in selected patients with symptomatic carotid stenosis. This preliminary study in a limited series of patients with unstable carotid plaques revealed that endovascular treatment has a satisfactory outcome considering the very high risk profile of the patient population. The evaluation of some biomarkers suggested an inflammatory role in the process of an unstable carotid plaque generating an acute cerebral event.*

Keywords: Symptomatic carotid stenosis; Deferred urgency carotid artery stenting; Unstable carotid plaque; Vulnerability biomarkers.

Introduction

The role of early carotid revascularization in symptomatic patients with severe carotid stenosis is still unclear. Although the pooled analyses from the European Carotid Surgery Trial (ECST) and the North American Symptomatic Carotid Endarterectomy Trial (NASCET)[1] clearly showed that the benefit from carotid endarterectomy (CEA) is *maximal* in symptomatic patients operated on within 2 weeks of the index event, most specialists involved in stroke care are reluctant to undertake carotid revascularization immediately after the onset of stroke for fear that hemorrhagic transformation of the cerebral infarct will occur.[2–7]

Delaying intervention quite probably means that patients are better selected, and this could guarantee better early outcomes, but this delay can also result in an interval stroke rate of 9–15%.[8]

The real goal of early intervention is to stop the plaque embolization from a vulnerable lesion at the carotid bifurcation, which is the most common pathogenic mechanism for cerebral ischemia from carotid atherosclerotic disease. However, the challenge of recognizing vulnerable plaques noninvasively at early stages, and before the onset of an acute clinical event, still remains such.

A number of studies have demonstrated that atherosclerosis is clearly an inflammatory disease and

*Corresponding author. C. Setacci, Vascular and Endovascular Surgery Unit, University of Siena, Viale Bracci, 53100 Siena, Italy.
E-mail address: setacci@unisi.it

1078–5884/000644 + 08 $34.00/0

does not result simply from the accumulation of lipids.[9–11] These studies have also correlated different biomarkers with cerebrovascular and cardiovascular disease. However there are as yet no specific biomarkers that allow accurate identification.

The aim of this prospective observational registry, called SUBMARINE (**S**erum And **U**rinary Plaque Vulnera**b**ility Bio**mar**kers Detec**t**io**n** Before And After Carotid St**e**nt Implantation) was to study the outcome of symptomatic patients with recent first or recurrent TIA or minor stroke, bearing severe carotid artery stenosis and submitted to early percutaneous treatment by stenting with cerebral protection using a single device (Accunet™ filter and Acculink™ stent – Abbott Vascular Devices, Redwood City, CA, USA).

The primary end-point was the absence of cerebrovascular events at discharge.

Secondary end-points were: (a) the assessment of death rate, combined stroke and death rate, and any new neurological event (including TIA and any stroke) at 30 days; (b) the evaluation of biological activity of symptomatic carotid plaques by serial serum and urinary markers, before and after treatment.

Methods

The study was conducted at 8 centers. From May 2005 to June 2006, 57 patients (37 males, 65%; mean age 76.7 ± 8.0 years) were enrolled in this prospective registry.

Inclusion criteria were a recent (within 24 hours) acute hemispheric event, including minor stroke, single transient ischemic attack (TIA), or recurrent (''crescendo'') TIA, in the vascular territory of a diseased carotid artery with documented ultrasonographic stenosis $\geq 50\%$. The target lesion stenosis was measured by the surgical team using the NASCET criteria.[12]

Minor stroke was defined as any new neurological deficit that persisted for >24 hours, associated with a modified Rankin[13] score of less than 3 (i.e. at most only slight disability from the index stroke, without the need for assistance in daily affairs).

The following patients were excluded from the study: those with a modified Rankin scale ≥ 3, with evidence of potential alternative sources of emboli, such as chronic atrial fibrillation or malformation of the circle of Willis, either with lacunar infarct (LACS, *Lacunar Syndromes*) or infarct in the vertebrobasilar territory (POCS, *Posterior Circulation Syndromes*), with images suggesting the presence of a thrombus in the common or internal carotid artery (string sign) and/or slow flow during contrast injection into the carotid axis.

The study was approved by the Ethics Committee and informed consent was obtained from all potential study candidates prior to any procedure.

Baseline evaluation involved medical history, neurological history, carotid duplex imaging, CT scan and blood, serum and urine collections to detect inflammatory biomarkers. A complete neurological examination (modified Rankin Score,[13] NIH Stroke Scale – NIHSS[14]) was performed for each patient pre-procedure, post-procedure, at discharge and at 30 days follow-up.

In order to evaluate the outcome of revascularization, the patient sample was divided into clinically improved (decrease on NIHSS ≥ 2), those unimproved (unchanged NIHSS or decrease on NIHSS < 2), and those who were impaired (increase on NIHSS).

A critical event committee composed of two neurologists evaluated the neurological history at the different time intervals of the study and judged any neurological adverse events.

Target lesion morphology with respect to ulceration, calcification and eccentricity was assessed by ultrasound and intra-operative angiography. A lesion comprising $\geq$02 craters of $\geq$03 mm in depth or with poorly defined edges and a hazy appearance was defined as ulcerated, while a radiopaque area under fluoroscopy was defined as calcified.

The stenting procedure was performed within 24–48 hrs of the last attack in patients with TIA and between 14 and 30 days in patients with stroke, in order to obtain the stabilization of the cerebral ischemic lesion.

Prior to surgery all patients received 100–300 mg/d of aspirin for at least 2 days prior to the operation and either 75 mg of Clopidogrel for at least 24 hours prior to the operation, or 300 mg of Clopidogrel on the day of the surgery, at least 4 hours before commencing the procedure. Heparin was administered during the procedure at a dose of 5,000 IUs or similar, so that an ACT > 250 sec was maintained throughout the operation. Following surgery, all patients were given 75 mg/d of Clopidogrel for a period of 1 month, and 100 mg/d of aspirin indefinitely.

CAS was performed according to each unit's existing standards of care. The vascular system was accessed via the femoral artery. Angiography of the diseased carotid artery was performed to confirm eligibility criteria. An appropriate guide catheter or long sheath was advanced over the guidewire to engage the ostium of the diseased carotid artery. The guidewire with the filter was gently advanced across and through the lesion and the filter was then positioned in the distal segment of the internal carotid artery. If

the filter did not cross the lesion easily, careful predilatation was performed. A stent of appropriate size and length, according to the investigator's discretion, was then placed at the lesion site. A post-dilatation balloon was carefully advanced until it was positioned within the stent and then dilated to pressures sufficient only to appropriately expand the stent. Angiography of the stented segment was then repeated to ensure the adequacy of stent deployment, lesion dilatation, and distal flow.

The histo-morphometric analysis of plaque debris was performed on each of the filters used. After removal, filters were immediately fixed in 10% buffered formalin and sent to the external histology core laboratory for histological and cytological analysis. The investigators who performed the histological and cytological assessment were unaware of the angiographic and procedural results. Morphological identification of particles was done at 100 and 200-fold magnification according to the following criteria: (1) a particle was identified as a thrombus fragment if it appeared to be a conglomerate of erythrocytes with embedded strands of fibrin, and if the structure of single erythrocytes could not be discerned; (2) a particle was identified as a plaque fragment if it was a conglomerate of erythrocytes with embedded strands of fibrin and localized structures corresponding to cholesterol clefts, with the contour either serrated or smooth but clearly different from a fissure. Lipid-laden foam cells confirmed the diagnosis of a plaque; (3) a particle was defined as an intima fragment if it consisted of small pieces of collagen fibers covered by endothelial cells or attached to plaque fragments. These structures could be embedded in fibrin or thrombus fragments, and foam cells could also be included.

The carotid plaque vulnerability markers studied were: pregnancy-associated protein A (*PAPP-A*), high sensitivity C-reactive protein (*hsCRP*), Interleukin 6 (*IL-6*), Interleukin 8 (*IL-8*), proteolytic enzyme metalloproteinases (*MMP2* and *MMP9*), tumour necrosis factor (*TNF*) and CD40 ligand (*CD40L*). These vulnerability markers were evaluated prior to stenting, immediately afterwards and at the 30 day follow-up.

Blood was taken by venipuncture, placed in an EDTA-free tube and centrifuged within 30 minutes of collection at 3000 g $\times$ 20 min, then the serum was carefully separated from blood corpuscolate elements. The serum was stored at -20° for core laboratory determination of vulnerability biomarkers. Urine testing was only done for PAPP-A levels: urine was collected in a plastic vial (10 cc) and stored at -20° until ready to be sent to the core laboratory for measurements. At the core laboratory serum and urinary PAPP-A levels were determined by means of a biotin−tyramide−amplified enzyme immunoassay with a detection limit of 0.03 m IU per litre and intraassay and interassay coefficients of variation of 10 percent and 15 percent, respectively. PAPP-A polyclonal antibodies were used for capture and a combination of monoclonal antibodies was used for detection. A highly sensitive latex particle−enhanced immunoturbidimetric assay was used to quantify the level of high sensitivity C-reactive protein.

Other serum biomarkers (IL-6, IL-8, CD-40L, MMP-2, MMP-9, TNF-alpha) were measured by enzyme-linked immunosorbent assay (*ELISA*), using commercially available immunoradiometric kits (Pantec, Turin, Italy).

Statistical analysis

Categorical data were presented as absolute frequencies and percent values. Quantitative measurements were expressed as mean $\pm$ SD. Laboratory parameters assessed at the beginning and at the end of the observation period were evaluated by parametric (Student's t test for paired data) or non parametric (Wilcoxon's test for paired data) methods. Comparison between biomarkers levels at different study intervals was performed by one-way repeated measures analysis of variance (ANOVA) for parametric parameters. A p value ≤ 0.05 was considered statistically significant (statistical package: BMDP New System 2.0 − by Statistical Solutions Ltd, Cork − Ireland).

Results

Of the 57 patients enrolled, 42.1% ($n = 24$) had first episode or recurrent TIA, 57.9% ($n = 33$) had minor stroke. The demographic characteristics are shown in Table 1.

The target lesion stenosis was 75.9 $\pm$ 11.6%. Lesion characteristics are shown in Table 2.

Successful stent implantation was achieved in all cases (100%). An Acculink™ stent was implanted in 96.5% of patients and a different type of stent (Carotid Wallstent − Boston Scientific Corp, Natick, MA, USA) was implanted in the other 2 patients (3.5%).

No intra-procedural neurological complications occurred, while the minor complications that occurred during the procedure were ICA vasospasm in 14% of cases ($n = 8$) and severe hypertension in one case (1.7%). Procedural success, defined as absence of new cerebrovascular events at discharge (including major stroke, minor stroke, or TIA), was assessed at 96.5%.

Table 1. Main baseline demographic characteristics and risk factors profiles

	($n = 57$)	
Age [Years (mean ± S.D.); range (Min−Max)]	76.7 ± 8.0	57−93
Male (*n*; %)	37	64.9
Diabetes Total (*n*; %)	13	22.8
Diabetes Non ID (*n*; %)	10	17.5
Hypertension (*n*; %)	49	86.0
Hypercholesterolemia (*n*; %)	15	26.3
HDL < 35 (*n*; %)	24	42.1
Hypertrigliceridemia (*n*; %)	7	12.3
Coronary artery disease (CAD) (*n*; %)	13	22.8
Previous MI (*n*; %)	8	14.0
Previous PTCA (*n*; %)	4	7.0
Previous CABG (*n*; %)	5	8.8
Family History for CAD (*n*; %)	15	26.3
Peripheral artery disease (*n*; %)	3	5.3
Renal Insufficiency (BUN > 20 mg/dl) (*n*; %)	6	10.5

ID: *insulin dependent*; HDL: *high density lipoprotein*; MI: *myocardial infarct*; PTCA: *percutaneous transluminal coronary angioplasty*; CABG: *coronary artery bypass graft*; BUN: *blood urea nitrogen*.

Two patients experienced an in-hospital new neurological event (TIA, 3.5%). Other *non neurological* adverse events were 1 persistent bradicardia (1.7%), 1 patient with non-Q wave AMI (1.7%) and 1 with renal insufficiency (1.7%).

At 1 month total neurological adverse events included 1 death (1.7%) and 2 TIAs (3.5%). The sudden death that occurred during the 30-day follow-up period was probably due to a myocardial infarction.

The NIH/Rankin Score at the different study intervals is shown in Fig. 1. No patients presenting with minor stroke experienced a worsening of the neurological deficit at hospital discharge. The postoperative NIHSS score at hospital discharge showed that in 50.9% (29/57) of patients the neurologic deficit remained unchanged (decrease in NIHSS < 2) and in 28/57 (49.1%) the NIHSS score improved (decrease in NIHSS ≥ 2). At 1 month 32/56 patients (57.1%) experienced an improvement in their initial neurologic deficit, while in 42.9 % of patients the deficit remained stable ($p > 0.05$ in the neurological changes at different study intervals).

Table 2. Lesion characteristics

	($n = 57$)	
Hyperechoic lesions (*n*; %)	30	52.6
Hypoechoic lesions (*n*; %)	27	47.4
Angiographic ICA stenosis in % (Mean ± SD; Range)	75.9 ± 11.6	60−99
Ulcerated lesion (*n*; %)	7	12.2
Calcific lesion (*n*; %)	16	28.0
Eccentric lesion (*n*; %)	15	26.3
CCA involvement (*n*; %)	24	42
ECA involvement (*n*; %)	14	25
Angiographic Controlateral ICA stenosis in % (Mean ± SD; Range)	40.0 ± 34.4	0−100

ICA: *internal carotid artery*; CCA: *common carotid artery*; ECA: *external carotid artery*.

The data concerning plaque vulnerability biomarkers are shown in Figs. 2−4 (serum markers) and Table 3 (urinary marker). *P*-values, statistical significance and confidence intervals are indicated only in the case of significance during the different time intervals of the study: Pre-procedure (Pre), Post-procedure (Post) and 30 day Follow-up (FU).

Vulnerability markers, in particular those reflecting an active systemic inflammatory process as PAPP-A, hs-CR, and inflammatory cytokines such as IL-6 and IL-8, were significantly elevated at the time of enrolment, increased after stenting and decreased after 30 days. A remarkable variation was noted for the PAPP-A level, which decreased from 15.1 m IU/L after stenting to 6.9 m IU/L at 30 days, $p < 0.01$ CI (95% confidence interval) 1.8−15.3; for hs-CRP, which decreased from 23.2 mg/L after stenting to 9.7 mg/L at 30 days, $p < 0.01$ 95% CI 6.7−19.9; and for IL-6, which decreased from 13.5 pg/ml after stenting to 7.4 pg/ml at 30 days, $p < 0.01$ 95% CI 3.6−9.1.

The data concerning histopathologic evaluation of plaque debris collected within the filters are summarized in Table 4.

Discussion

Effective and early management of patients with acute symptoms due to carotid stenosis is still the subject of debate. The inability to predict who is at higher early risk of a recurrent stroke after a cerebrovascular event (TIA or stroke) may explain the variation in management of acute stroke comparing physician to physician and institution to institution.

CEA has traditionally been delayed for 4 to 8 weeks because of fear of hemorrhagic transformation of the ischemic infarct. However, this disapproval of early treatment after an acute stroke is inappropriate. Rothwell et al[1] have recently analyzed pooled data from the European Carotid Surgery Trial and North American Symptomatic Carotid Endarterectomy Trial (a total of 5893 patients). The analysis of long-term stroke prevention (per 1000 CEAs at 5 years) in relation to the delay in surgery clearly showed that benefit from surgery decreased rapidly with time elapsed since the last neurological symptoms. Profit from endarterectomy seems to depend not only on the degree of carotid stenosis, but also on delay in surgery after the presenting event, and the conclusion was that the procedure should ideally be done within 2 weeks of the patient's last symptoms. The same author in 2007 has reported a prospective study[15] that shows

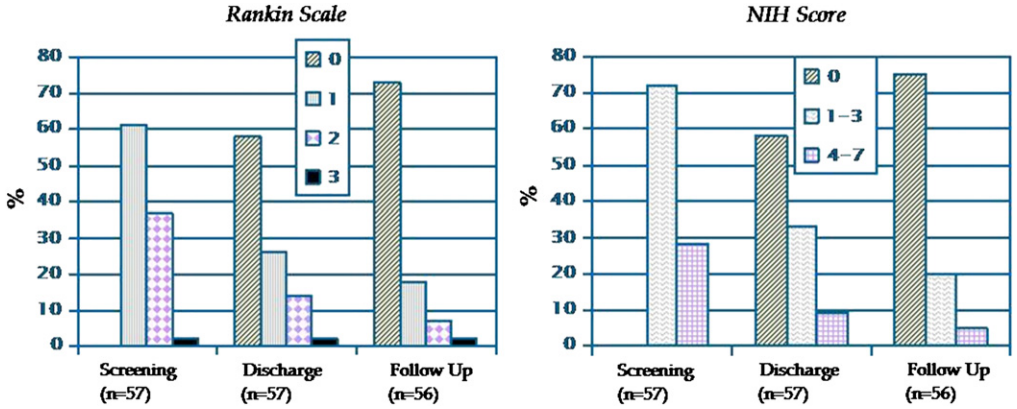

Fig. 1. Rankin Score/NIH at different study intervals.

how an early treatment (medical and/or surgical) of all patients presenting with TIA or minor stroke can prevent about 80% of early recurrent stroke.

In a prospective study of patients presenting with acute symptomatic high-grade internal carotid artery stenosis, the Oxford Vascular Study Group[16] recently observed that for patients with TIA the 7 day, 30 day and 3 month risks of stroke were 8%, 12% and 17% respectively, while for patients presenting with minor stroke (NIH stroke score < 3) parallel data were 12%, 15% and 19%. These data suggest that acute stroke or TIA be considered as medical emergencies that require rapid evaluation and rapid targeting of treatment. The reported stroke risks highlight how early removal of the carotid plaque that is considered to be the embolic source could be crucial in stroke prevention.

An Italian multicenter study – Surgical Treatment of Acute Cerebral Ischemia (STACI)[17] – has recently shown that patients whose neuroimaging studies document a recent, limited cerebral infarction in the early hours after a stroke can safely undergo very early CEA (1.5 days after the stroke). This study underlines that if patients are strictly selected for early CEA after an acute stroke, early surgery incurs similar risks to elective surgery.

The SPREAD guidelines[18] also clearly state that carotid surgery is recommended as early as possible – within 2 weeks of the event – for patients with TIA, minor stroke or stabilized neurological deficit with normal CT scanning or minimal lesions (Grade A recommendation); while early carotid endarterectomy is not recommended for patients with disabling stroke or large infarction and/or brain oedema on CT scanning (Grade C recommendation).

With growing experience in endovascular treatment, CAS has been proposed as an alternative to CEA, but data regarding the outcome of patients with acute stroke undergoing urgent endovascular surgery are still scarce.

The main concern about CAS in urgent cases is that while with CEA the plaque is completely removed, after stenting it is only remodelled and its stabilization is essential to avoid later embolic events.

Our prospective registry was designed not only to demonstrate the safety and the efficacy of early CAS after TIA (within 24–48 hours) or minor stroke (within 14–28 days), but also to investigate the role of the stent as a plaque stabilizer by analysing the behaviour of vulnerability biomarkers during the postoperative period.

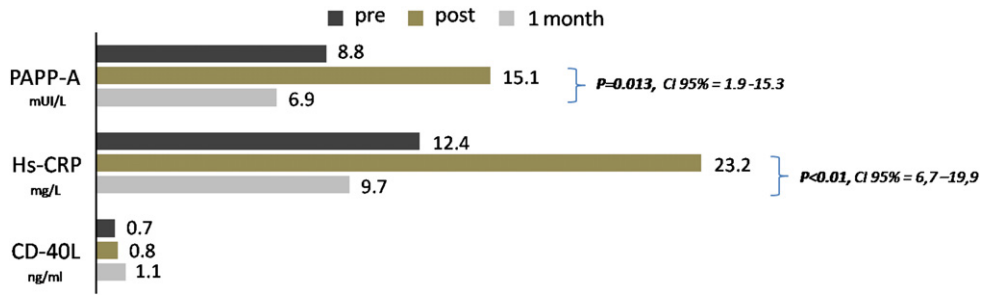

Fig. 2. PAPP-A, hs-CRP and CD-40L serologic levels at different time intervals.

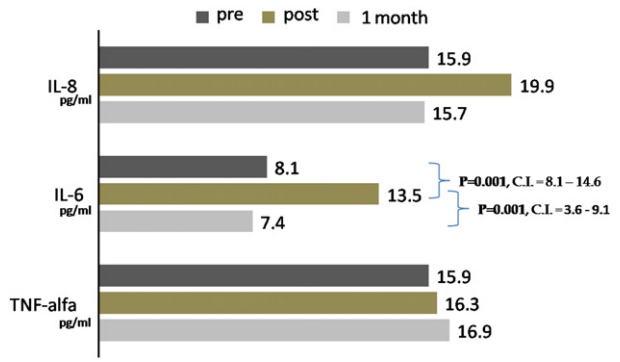

Fig. 3. IL-6, IL-8 and TNF-alpha serologic levels at different time intervals.

We demonstrated the safety of CAS in this series of high risk/complex carotid cases enrolled and treated soon after the onset of symptoms, as shown by the absence of neurological complications during the procedure, a high procedural success rate, and the very low rate of minor cerebrovascular events in the postoperative period. Moreover, the high rate of significant debris in the filter at histo-morphometric analysis (54/57, 94.7%) may suggest the utility of a cerebral protection device in such symptomatic cases.

The clinical outcome at 1 month of a 1.7% stroke/mortality rate (1 non-neurological death) and 3.5% rate for all neurological events (2 post-procedure TIAs) are extremely satisfactory considering the very high risk profile of the patient population.

In this study an open cell stent was used in 55 out of 57 cases. Moreover, we are aware of the important role of scaffolding of the embolic plaque by the struts of the stent.[19] Our purpose in the near future is to further investigate the safety of CAS in symptomatic stroke patients using either a closed or an open cell stent (Submarine II Registry).

Although the central role of inflammation in the pathogenesis of atherosclerosis has been demonstrated,[20–28] the difficulty of noninvasively recognizing

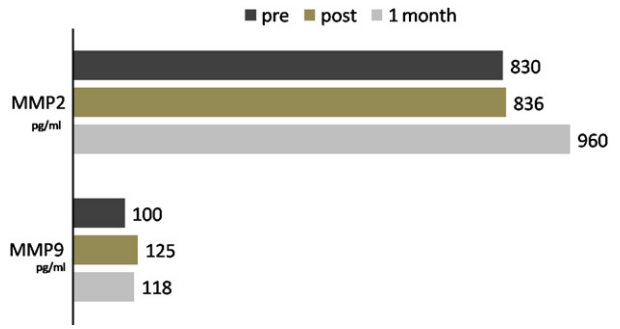

Fig. 4. MMP-2 and MMP-9 serologic levels at different time intervals.

Table 3. Urine Plaque vulnerability biomarkers at different time intervals of the study

PAPP-A mIU/l	Overall ($n = 57$)	TIA ($n = 24$)	Minor Stroke ($n = 33$)
Pre-procedure	1.02 ± 0.9	1.01 ± 0.8	1.02 ± 1.02
Post-procedure	1.21 ± 0.86	1.22 ± 0.78	1.21 ± 0.93
p-value	>.05	>.05	>.05

vulnerable plaques at early stages, and before the onset of an acute clinical event, still remains. Several studies have shown that the histomorphologic composition of the atherosclerotic plaque,[29–31] as well as the degree of stenosis, is a major determinant for increased risk of carotid plaque disruption and cerebral embolization. In our Registry the vulnerability markers, in particular those reflecting an active inflammatory infiltrate of the plaque, such as PAPP-A, hs-CR, MMPs, and inflammatory cytokines, such as IL-6 and IL-8, were significantly elevated in all patients at the time of enrolment. Several of these biological markers have already been proposed to identify both ruptured and eroded unstable coronary plaques.[32–35] Sangiorgi et al[36] have recently evaluated cellular PAPP-A expression in stable and unstable carotid atherosclerotic plaques collected from patients undergoing surgical carotid endarterectomy. Their results clearly correlate vulnerability biomarker levels with histopathologic carotid plaque complexity: PAPP-A was significantly higher in patients with vulnerable and ruptured plaques (respectively 7.43 ± 0.97 m IU/L and 6.97 ± 0.75 m IU/L) compared to patients with stable plaques (4.02 ± 0.18 m IU/L).

In our study the elevated serum PAPP-A values (8.82 ± 12.1 m IU/L) at the time of enrolment revealed the systemic evidence of the presence of an inflammatory state, possibly directly related to the carotid plaque in our series of symptomatic patients. This study is the first to confirm that complex, high-risk plaques are associated with an increase in biomarkers of vulnerability. Some of these markers, such as serum levels of PAPP-A, Hs-CRp and IL-6, increased significantly after the procedure and decreased at follow-up. Although we are aware that this trend is potentially influenced by many other factors independently of the placement of a carotid stent (such as medication, control of cardiovascular risk factors etc.), and may be due to systemic effects, we believe that our study is the first in the literature to show a possible correlation between the behavior of some serum markers and stent implantation at the level of unstable carotid plaques. However, the behavior of other biomarkers (serum MMP, CD-40L, TNF-alpha, and urinary PAPP-A) seemed to be of no value in our investigation.

Table 4. Histopathologic evaluation of plaque debris collected within the filters

	Overall	TIA	Minor Stroke	*P*-value
Thrombus fragment (score 1)	19 (33,3%)	7 (29.2%)	12 (36.4%)	0.894*
Plaque fragment (score 2)	18 (31,6%)	8 (33.3%)	10 (30.3%)	
Intima fragment (score 3)	17 (29,8%)	7 (29.2%)	10 (30.3%)	
Absent	3 (5,3%)	2 (8.3%)	1 (3.0%)	
Total	57 (100.0%)	24 (100.0%)	33 (100.0%)	

* *P*-value concening Chi-square test on (Minor Stroke and TIA).

On the basis of these considerations, our hypothesis is that selected serum biomarkers, such as PAPP-A, Hs-CRp and IL-6, could be related to the vulnerability of symptomatic atherosclerotic plaques and that carotid plaque stabilization by means of mechanical intervention may also be linked to a reduction in the expression of these vulnerability markers.

At this time further investigations are needed to verify our hypothesis and to validate the utilization of these vulnerability biomarkers as enhanced tools in diagnostic pathways for symptomatic and asymptomatic patients with carotid atherosclerotic disease.

At present, sophisticated imaging techniques such as pixel density analysis and elastography at Duplex examination,[37,38] magnetic resonance imaging for tissue characterization,[39] or local temperature probes[40,41] all hold promise for the non-invasive identification of vulnerable plaques and the detection of silent atheroma.

In conclusion, our study demonstrated that early treatment with protected carotid stenting is both feasible and safe in selected patients with first episode or recurrent TIA or minor stroke. This preliminary study in a limited series of patients revealed that an urgent endovascular approach has a satisfactory outcome considering the very high risk profile of the patient population.

The time course of some serologic markers may be of great interest in the study of the behaviour of unstable carotid plaques following stent implantation, and could merit the attention of further studies.

Acknowledgements

The Authors take great pleasure in thanking the critical neurological event committee with special regards to Dr. Menegazzo and Dr. Sessa, for performing the systematic review of neurological data and providing substantial support to the data analysis. The Authors gratefully acknowledge the histopathological evaluation of carotid filters and expert data collection of plaque debris conducted by Dr. Mauriello.

Appendix A.

Participating centers

1. Vascular and Endovascular Surgery − University of Siena − Siena, Italy − Dr Setacci.
2. Vascular Surgery − University of Bologna − Bologna, Italy − Dr Stella.
3. Cardiology Department, Mirano Hospital, Mirano (Venice), Italy − Dr Reimers.
4. Invasive Cardiology Unit, San Raffaele Scientific Institute, Milan, Italy − Dr Sangiorgi.
5. Vascular Surgery − Ferrarotto Hospital − Catania, Italy − Dr. Monaca.
6. Neuroradiology Unit − SS. Annunziata Hospital − Taranto, Italy − Dr. Burdi.
7. Interventional Unit − Pavia, Italy − Dr. Calabrese
8. Vascular Surgery Unit − Cardarelli Hospital − Naples, Italy − Dr. Ruotolo.

References

1 Rothwell PM, Eliasziw M, Gutnikov, Warlow CP, Barnett HJM, for the Carotid Endarterectomy Trialists' Collaboration. Endarterectomy for symptomatic carotid stenosis in relation to clinical subgroups and timing of surgery. *Lancet* 2004;**363**:915−924.
2 Gasecki AP, Ferguson GG, Eliasziw M, Clagett GP, Fox AJ, Hachinski V *et al*. Early endarterectomy for severe carotid artery stenosis after a non-disabling stroke: results from the North American Symptomatic Carotid Endarterectomy Trial. *J Vasc Surg* 1994;**20**:288−295.
3 Sacco RL, Foulkes MA, Mohr JP, Wolf PA, Hier DB, Price TR. Determinants of early recurrence of cerebral infarction. The Stroke DataBank. *Stroke* 1989;**20**:983−989.
4 Dosick SM, Whalen RC, Gale SS, Brown OW. Carotid endarterectomy in the stroke patient: computerized axial tomography to determine timing. *J Vasc Surg* 1985;**2**:214−219.
5 Meyer FB, Sundt Jr TM, Piepgras DG, Sandok BA, Forbes G. Emergency carotid endarterectomy for patients with acute carotid occlusion and profound neurological deficits. *Ann Surg* 1986;**203**:82−89.
6 Pritz MB. Timing of carotid endarterectomy after stroke. *Stroke* 1997;**28**:2563−2567.
7 Gertler JP, Blankensteijn JD, Brewster DC, Moncure AC, Cambria RP, Lamuraglia GM *et al*. Carotid endarterectomy for unstable and compelling neurologic conditions: do results justify an aggressive approach? *J Vasc Surg* 1994;**19**:32−40.
8 Paty PS, Darling 3rd RC, Feustel PJ, Bernardini GL, Mehta M, Ozsvath KJ *et al*. Early carotid endarterectomy after acute stroke. *J Vasc Surg* 2004;**39**:148−154.
9 Ross R. Atherosclerosis − an inflammatory disease. *N Engl J Med* 1999;**340**:115−126.
10 Qiao J-H, Tripathi J, Mishra NK, Cai Y, Tripathi S, Wang XP *et al*. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. *Am J Pathol* 1997;**150**:1687−1699.
11 Golledge J, Greenhalgh RM, Davies AH. The symptomatic carotid plaque. *Stroke* 2000;**31**:774−781.
12 North American Symptomatic Carotid Endarterectomy Trial (NASCET) Collaborators Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med* 1991;**325**:445−453.

13 Bonita R, Beaglehole R. Modification of Rankin Scale: Recovery of motor function after stroke. *Stroke* 1988;**19**:1497–1500.
14 Stroke scales and clinical assessment tools. NIH Stroke Scale (NIHSS). Available from: http://www.strokecenter.org/trials/scales/nihss.html.
15 Rothwell PM, Giles MF, Chandratheva A, Marquardt L, Geraghty O, Redgrave JN *et al*. Early use of Existing Preventive Strategies for Stroke (EXPRESS) study. Effect of urgent treatment of transient ischaemic attack and minor stroke on early recurrent stroke (EXPRESS study): a prospective population-based sequential comparison. *Lancet* 2007;**370**:1432–1442.
16 Coull AJ, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004;**328**:326–328.
17 Sbarigia E, Toni D, Speziale F, Acconcia MC, Fiorani P. Early carotid endarterectomy after ischemic stroke: the results of a prospective multicenter Italian study. *Eur J Vasc Endovasc Surg* 2006; **32**:229–235.
18 SPREAD. Stroke prevention and educational awareness diffusion. 2007. Available from: http://www.spread.it/
19 Bosiers M, de Donato G, Deloose K, Verbist J, Peeters P, Castriota F *et al*. Does free cell area influence the outcome in carotid artery stenting? *Eur J Vasc Endovasc Surg* 2007;**33**: 135–141.
20 Brown B, Zhao X, Sacco E, Alberts J. Lipid lowering and plaque regression: new insights into prevention of plaque disruption and clinical events in coronary disease. *Circulation* 1993;**87**: 1781–1791.
21 Frink RJ. Chronic ulcerated plaques: new insight into the pathogenesis of acute coronary disease. *J Invasive Cardiol* 1994; **6**:173–185.
22 Falk E, Shah PK, Fuster V. Coronary plaque disruption. *Circulation* 1995;**92**:657–671.
23 Crea F, Biasucci LM, Buffon A, Liuzzo G, Monaco C, Caligiuri G *et al*. Role of inflammation in the pathogenesis of unstable coronary artery disease. *Am J Cardiol* 1997;**80**:10–16.
24 Boyle JJ. Association of coronary plaque rupture and atherosclerotic inflammation. *J Pathol* 1997;**181**:93–99.
25 Pasterkamp G, Schoneveld AH, Van Der Wal AC, Hijnen DJ, Van Wolveren WJ, Plomp S *et al*. Inflammation of the atherosclerotic plaque and shoulder of the plaque is a common and locally observed feature in unruptured plaques of femoral and coronary arteries. *Arterioscler Thromb Vasc Biol* 1999;**19**:54–58.
26 Ross R. Atherosclerosis: an inflammatory disease. *N Engl J Med* 1999;**340**:115–126.
27 Liuzzo G, Kopecky SL, Frye RL, O'Fallon WM, Maseri A, Goronzy JJ *et al*. Perturbation of T-cell repertoire in patients with unstable angina. *Circulation* 1999;**100**:2135–2139.
28 Blake GJ, Ridker PM. Inflammatory mechanisms in atherosclerosis: from laboratory evidence to clinical application. *It Heart J* 2001;**2**:796–800.
29 Carr S, Farb A, Pearce WH, Virmani R, Yao JS. Atherosclerotic plaque rupture in symptomatic carotid artery stenosis. *J Vasc Surg* 1996;**23**:755–766.
30 Carr SC, Farb A, Pearce WH, Virmani R, Yao JST. Activated inflammatory cells are associated with plaque rupture in carotid artery stenosis. *Surgery* 1997;**122**:757–764.
31 Spagnoli LG, Mauriello A, Sangiorgi G, Fratoni S, Bonanno E, Schwartz RS *et al*. Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke. *JAMA* 2004;**292**: 1845–1852.
32 Loftus IM, Thompson MM. The role of matrix metalloproteinases in vascular disease. *Vasc Med* 2002;**7**:117–133.
33 Loftus IM, Naylor AR, Bell PR, Thompson MM. Matrix metalloproteinases and atherosclerotic plaque instability. *Br J Surg* 2002; **89**:680–694.
34 Beaudeux JL, Giral P, Bruckert E, Bernard M, Foglietti MJ, Chapman MJ. Serum matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 as potential markers of carotid atherosclerosis in infraclinical hyperlipidemia. *Atherosclerosis* 2003;**169**:139–146.
35 Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, Christiansen M, Holmes Dr JR *et al*. Pregnancy associated plasma protein A as a marker of acute coronary syndromes. *N Engl J Med* 2001;**345**:1022–1029.
36 Sangiorgi G, Mauriello A, Bonanno E, Oxvig C, Conover CA, Christiansen M *et al*. Pregnancy-associated plasma protein-a is markedly expressed by monocyte-macrophage cells in vulnerable and ruptured carotid atherosclerotic plaques: a link between inflammation and cerebrovascular events. *J Am Coll Cardiol* 2006; **47**:2201–2211.
37 Lal BK, Hobson RW, Pappas PJ, Kubicka R, Hameed M, Chakhtoura EY *et al*. Pixel distribution analysis of B-mode ultrasound scan images predicts istologic features of atherosclerotic carotid plaque. *J Vasc Surg* 2002;**35**:1210–1217.
38 Cespedes EI, de Korte CL, van der Steen AF, von Birgelen C, Lancee CT. Intravascular elastography: principles and potentials. *Semin Interv Cardiol* 1997;**2**:55–62.
39 Shinnar M, Fallon JT, Wehrli S, Levin M, Dalmacy D, Fayad ZA *et al*. The diagnostic accuracy of ex vivo MRI for human atherosclerotic plaque characterization. *Arterioscler Thromb Vasc Biol* 1999;**19**:2756–2761.
40 Casscells W, Hathorn B, David M, Krabach T, Vaughn WK, Mcallister HA *et al*. Thermal detection of cellular infiltrates in living atherosclerotic plaques: possible implication for plaque rupture and thrombosis. *Lancet* 1996;**347**:1447–1451.
41 Verheye S, De Meyer GR, Van Langenhove G, Knaapen MW, Kockx MM. In vivo temperature heterogeneity of atherosclerotic plaques is determined by plaque composition. *Circulation* 2002; **105**:1596–1601.

Accepted 17 February 2008
Available online 25 March 2008